American Journal of Medical Genetics 65:226–234 (1996)

Segregation Analysis of Microcephaly

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Microcephaly is a heterogeneous disorder with genetic and environmental causes. However, there is little information on what proportion of cases are caused by inherited susceptibility, or the mode of inheritance in familial cases. To address these questions, we have performed classical and complex segregation analyses for microcephaly on 2 sets of family data collected from genetic counseling clinics in Vancouver and Jerusalem. These samples consisted of 143 affected individuals in 127 families ascertained from Vancouver, and 101 affected individuals in 59 families ascertained from Jerusalem. The results of the segregation analyses for the Vancouver sample indicated that approximately half of all microcephaly cases were due to highly penetrant recessive mutant alleles, with the remainder being sporadic. Although a recessive model allowing for the occurrence of sporadic cases fit the data from Vancouver best, a dominant model could not be statistically rejected. The classical segregation analysis on the Jerusalem sample suggested that both a dominant model with 29% of the cases being sporadic and a purely recessive model provided adequate fit to the data. Although the complex segregation analysis of this sample indicated that a dominant model provided a more parsimonious explanation for the observed familial variation, a recessive model was only marginally rejected. It should be noted that in the Jerusalem sample, families tended to be ascertained in the genetic counseling clinic only after the birth of a second affected child. This could be a potential bias which could inflate the segregation ratio, thus giving the impression of dominant inheritance. Our analyses, while confirming the complex nature of the cause of microcephaly, indicate that it may be necessary to await the results of genetic linkage analysis before a definitive mode of inheritance can be determined.

KEY WORDS: microcephaly, segregation analysis, heterogeneity

INTRODUCTION

Microcephaly is diagnosed in an individual whenever the occipitofrontal head circumference (OFC) is 2 or more standard deviations below the mean, given the age and gender of that individual. When present at birth, microcephaly is usually classified as primary, and secondary when the birth OFC is normal but microcephaly develops after birth. Microcephaly is also termed "pure" when there are no associated anomalies except those that can be attributed to the small size of the brain, such as spasticity or seizures; in contrast, complex or syndromal microcephaly is defined by additional developmental disorders occurring in organs and tissues other than the brain [Opitz and Holt, 1990]. Genetic and environmental factors are both known to cause microcephaly. Among the hereditary forms both recessive and dominant inheritance of microcephaly has been described in individual families, and these can occur as either nonsyndromal or syndromal cases, with or without mental retardation [Tolmie et al., 1987; Bawle and Horton, 1989; Teebi et al., 1987; Rossi et al., 1987]. For example, the recessively inherited Bloom syndrome phenotype sometimes includes microcephaly. Chromosomal aberrations also frequently lead to microcephaly, as for example that observed in Down syndrome or the 5p− syndrome [Schinzel, 1984]. Finally, several environmental factors leading to microcephaly have been identified, such as some teratogenic drugs and maternal phenylke-

Received for publication April 3, 1995; revision received January 10, 1996.

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tonuria [Opitz and Holt, 1990]. Although the contributions of single mutant genes, chromosomal aberrations and environmental factors to microcephaly have been demonstrated, two key questions remain unanswered. First, among genetic forms, does microcephaly occur purely as mendelian disorders, or, as a part of the phenotype of mendelian disorders, or is there multigenic inheritance as in other birth defects? Second, what are the relative contributions of genetic and non-genetic forms to microcephaly?

The frequency of microcephaly is highly variable and estimated to be between 1/1,360 and 1/93,000 livebirths [Tolmie et al., 1987]. The great variability in the overall frequency of microcephaly in different populations can be attributed to the variation in the frequency of the various causes in different populations. However, differences in the definition of microcephaly and in the ascertainment criteria utilized to obtain patients can also lead to the observed variation. The most direct way to resolve this difference is to estimate the frequency of genetic versus non-genetic forms in different populations, studied with similar phenotype definitions and ascertainment criteria. We attempt such an analysis in this study. Genetic studies on samples of microcephalic patients, ascertained through mentally retarded patients, were performed by Opitz and Holt [1990] and by Herbst and Baird [1982], on samples of 92 and 98 affected individuals, respectively. Tolmie et al. [1987] and Sujatha et al. [1989] studied 48 and 118 microcephalic patients, respectively, who had been referred to medical genetic services. In spite of these genetic studies on microcephaly, with the exception of Sujatha et al. [1989], no formal segregation analyses have been attempted to define the mode of inheritance of microcephaly and to assess the relative contributions of genetic versus environmental factors. For a group of primary microcephalics and assuming a purely genetic model, Sujatha et al. [1989] estimated a segregation ratio of 13% from 27 non-inbred families and a higher segregation ratio of 25% from 34 consanguineous families. These data imply the existence of rare recessive genes for primary microcephaly. The reason for the lower segregation ratio in non-inbred families is unclear, but could be due to the presence of non-genetic forms.

We present information on microcephalic patients and their families ascertained through the genetic counseling services in Vancouver and Jerusalem. The aims of this study were to compare the types of microcephaly observed in these two different populations and to estimate the frequency of causes, hereditary versus environmental, in all microcephalics where no clear environmental causes nor chromosomal aberrations were known to be responsible. To clarify the genetics of microcephaly, we performed classical and complex segregation analysis on a total of 186 nuclear families ascertained through a microcephalic child (proband). We tested specific models of genetic transmission and included the possibility of non-genetic, sporadic cases to study the inheritance pattern and relative contribution of genetic forms to the cause of microcephaly.

STUDY POPULATIONS AND METHODS

Study Populations

All patient files which listed a primary or secondary diagnosis of microcephaly in the genetic counseling clinics of the University Hospital (Shaughnessy site), University of British Columbia, Vancouver, Canada and the Hadassah University Hospital, Jerusalem, Israel were investigated. In 1990, when this study was initiated, there were approximately 33,000 and 8,000 patient files in the genetics services in Vancouver and Jerusalem, respectively, and these had been accumulated from 1960 and from 1964, respectively. Only records of families with at least one liveborn offspring affected with microcephaly and a known total number of liveborn offspring were utilized. From the Vancouver and Jerusalem clinics, 132 and 62 such families were identified, respectively. All families were ascertained through a single proband except for 3 families in Vancouver and 7 families in Jerusalem which had 2 probands each, and 1 family in Jerusalem which had 3 probands. When the mother had more than one spouse, only the offspring of the father of the affected child were used in the analyses. Families in which the microcephaly could be explained by suspected environmental exposure or by a known chromosomal aberration were not included in the sample. In the Vancouver sample there were 2 children affected with suspected environmental factors: one newborn infant had been exposed to intrauterine cytomegalovirus and another had severe hypoxia and brain damage at birth. Among the remaining 130 and 62 families from Vancouver and Jerusalem, karyotypes were available on 109 and 34 families, respectively. In each sample, 3 probands with constitutional chromosomal aberrations were observed: in Vancouver, 2 affected children had a ring 13 chromosome and one had an 18q− karyotype; in Jerusalem, there was one affected child each with a ring 15 chromosome, 5p− and 11p− karyotypes. In these 6 families, the proband was the only affected child. By excluding these cases, the Vancouver and the Jerusalem samples contained 127 and 59 families, respectively, and these were included in all subsequent analyses. Details of our study samples are provided in Table I. It should be noted that the patients investigated by Herbst and Baird [1982] were collected from the Vancouver area, and some of their patients may overlap with the patients in our sample.

TABLE I. Characteristics of Ascertained Microcephaly Families

Characteristic	Study site	
	Vancouver	Jerusalem
Number of families	132	62
Suspected cause		
Environmental	2	0
Chromosomal	3	3
Number of families analyzed	127	59
Number of probands	130	68
No. of multiplex sibships	12 (9%)	23 (39%)

Classical Segregation Analysis

The family data were first analyzed by the method of classical segregation analysis with ascertainment corrections [Morton, 1959], and used the computer program SEGRAN [Morton, 1969]. This analysis assumed that the familial aggregation of the trait is controlled only by a major locus and a contribution from non-genetic causes. Autosomal dominant, recessive and general (i.e., an arbitrary segregation ratio) major gene models were fit to the family data, with and without allowing for sporadic (non-genetic) cases. Under each assumed model, SEGRAN was used to calculate the likelihood of the sibship data conditional on the parental phenotypes; the ascertainment probability (π), proportion of sporadics (x), and the segregation ratio (P) were then estimated by the method of maximum likelihood. To judge the adequacy of fit of the estimated parameters for each model to the data, a likelihood ratio χ^2 was computed from the observed number of affected individuals as compared to that expected as calculated from the estimated parameters. This χ^2 was calculated from data grouped by sibship size and the number of affected individuals, to avoid the inclusion of cells with an expected number of 5 or less. The degrees of freedom for this test was calculated as the difference between the number of observed classes and the number of estimated parameters less one. The *P*-value for each hypothesis tested is provided, i.e., the probability of obtaining the observed χ^2 value or one larger by chance.

Complex Segregation Analysis

Complex segregation analysis was performed on the family data by using the mixed model of inheritance [Morton and MacLean, 1974] and the method of pointers [Lalouel and Morton, 1981], as implemented in the computer program POINTER [Lalouel and Yee, 1980]. For a dichotomous trait, this analysis assumed that familial aggregation of the trait was due to an underlying, but unobservable, liability scale (y) to which Mendelian inheritance of a single major gene (g), multifactorial transmission (c) and random environmental effects (e) contributed additively and independently: $y = g + c + e$. Affection status was defined by a threshold, Z, on the liability scale, such that all individuals with liability above Z were defined as affected. For a dichotomous trait, g is assumed to have mean 0 and variance G. The multifactorial transmissible and the environmental components were assumed to be normally distributed as N(0, C) and as N(0, E), respectively. Thus, the phenotypic variance was the sum of the three components, i.e., $V = G + C + E = 1$. The major locus had two alleles, M and m, with the disease allele, m, having a gene frequency q. The distance between the means of the two homozygous classes on the liability scale, t, is termed the displacement and measured in standard deviation units. The degree of dominance of the major gene, d, is defined such that t multiplied by d is the difference between the mean of the homozygous normal class and the mean of the heterozygous class on the liability scale. Thus, d = 0, ½, and 1 for recessive, codominant, and dominant mutant phenotypes.

Liability variation around each major genotype was assumed to be normally distributed and due to both multifactorial transmission, C, and random non-familial environmental effects, E. Heritability, h, was defined as C/V and was the proportion of familial phenotypic variance due to multifactorial effects. Because of possible effects of common sibling environment, C, and consequently h, may be numerically different in adults and children, so h values for adults and children were calculated separately, with z being the ratio of adulthood to childhood heritability. Phenotypic variance which could not be explained by either a major locus or multifactorial effects were assumed to be due to residual environmental variance, E.

For the POINTER analysis, the χ^2 of fit to a specific genetic hypothesis was calculated by taking the difference of the -2 ln(likelihood) values of a general hypothesis from a specific (nested) hypothesis. The degrees of freedom for these tests were calculated as the difference in the number of linearly independent estimated parameters in the two competing hypotheses. The ascertainment probability was estimated to be $\pi = 0.10$ from the distribution of probands in the families ascertained. For all segregation analyses, we assumed the incidence of microcephaly to be 1/10,000 livebirths, this being the geometric mean of the range of the observed incidences.

Genetic risks, or recurrence risks, were calculated for a given pedigree structure and the parameters estimated by maximum likelihood for each sample. For any given family structure, the next child was assumed to be either affected (a) or of unknown (u) phenotype. For each population, the -2 ln(likelihood) value was computed for the family with a further affected or unknown child using POINTER. The genetic risk was calculated as

$$risk = e^{(2\ln(L_a) - 2\ln(L_u))}$$

RESULTS

Population Description

Table II provides relevant phenotypic information on the families ascertained from the two populations. All families were ascertained through offspring and were nuclear families with average sibship sizes of 2.1 and 3.7 live births, in the Vancouver and Jerusalem families, respectively. The sex ratio among all live births in these families and among the affected offspring did not deviate significantly from 0.5 in either sample. The mental retardation status, known for 109 of 143 affected offspring in the Vancouver sample and for 91 of the 101 affected children in the Jerusalem sample, shows considerable variation between the two samples. Retardation status was stated as slight if the child was retarded and yet was able to read and walk, moderate if the child was able to walk but unable to read, and severe if the child was severely retarded and unable to walk. Table II shows the distribution of retarded microcephalic offspring in each sample. Although over 94% of the microcephalics were retarded, the severely retarded microcephalics comprised approximately 25% and 80% of the affected children with known retarda-

tion status in the Vancouver and Jerusalem samples, respectively. This difference is highly significant ($\chi^2 = 63.7$, 1 df, $P < 10^{-6}$) and is mirrored by the finding of an increase in the degree of mental retardation among the microcephalics in the Jerusalem, as compared to the Vancouver, sample. The reason for this large difference is unknown to us and may be due to a difference in the genes contributing to microcephaly in these two populations. In addition, social factors such as the educational status and socioeconomic status of the families may have an influence on the distribution of severely retarded microcephalics in these populations.

Twins were observed in both samples, all of whom were concordant for gender. Eight twin pairs (5 female and 3 male) were observed in 7 families in the Vancouver sample; these were among 267 liveborn, i.e., 1 twin pair per 33 live births. Two female twin pairs were unaffected; 1 in the family with 2 twin pairs. The other twin pair in this family were male twins, both of whom were affected. In the other 5 twin pairs, only one was affected and he was the only affected member in that sibship. In the Jerusalem sample, there were 2 twin pairs, both males, among 221 liveborn. There was one additional female twin pair, both of whom were stillborn. The frequency of twins in the Jerusalem sample was 1/110 live births, or, 1/73 if the stillborn twins were included. Among the liveborn twins, one pair was healthy while the other contained one affected member.

The 100% frequency of same sexed twins may indicate that there were more monozygotic twins among them than expected, although this is not confirmed. Among the 7 affected twin pairs (4 male and 3 female) the zygosity was known for 2 pairs: one pair was monoamniotic, while another was reported to be dizygotic. A third pair was monochorionic and diamniotic, which is most often an indication of monozygosity, but has been observed on rare occasion for dizygotic twins. Anderson et al. [1990] reported on 4 women who had multiple gestations with death of one of the fetuses in the second trimester and CNS damage to the other twin. Two of the damaged twins had microcephaly, one of them due to porencephaly. Anderson et al. reviewed the literature and concluded that the death in utero of one monochorionic twin presents a danger to the other twin, yet there was no information on the risk when the other fetus was miscarried.

OFC measurements were available for 21 of 254 parents in the Vancouver families and for 25 of 118 parents in the Jerusalem families. One mother in the Vancouver sample and 4 mothers in the Jerusalem sample had an OFC equal to or less than the 3rd percentile. The lack of fathers having an OFC in the 3rd percentile or less could be reflective of sampling bias or it could be suggestive of a parent-of-origin imbalance for microcephaly. However, a larger sample of parent-offspring pairs with OFCs at or below the 3rd percentile would be required to adequately address this hypothesis. It should be noted that, while the phenotype of interest in our study is microcephaly, the affected children were typically referred to the genetics clinics not just because of their small head size, but also because they had some degree of developmental delay and/or mental retardation. None of the parents observed to have OFCs in the 3rd centile or less were reported to have appreciable developmental delay or mental retardation. For this reason, these parents were considered to be unaffected for our analyses.

Table III provides information on the geographic origin of the families and their consanguinity. Among the 127 families sampled in Vancouver, the geographic origin of 99 was known. Of these, 86 were from the Americas and Europe, 11 from Asia or the Middle East (5 from China, 5 from India, and 1 from Kuwait) and 2 from Africa. Among the 59 families sampled from

TABLE II. Phenotype of Ascertained Microcephaly Families

Phenotype	Study site	
	Vancouver	Jerusalem
Number of families	127	59
No. liveborn offspring	267	221
Gender: Male	134 (51%)	97 (47%)
Female	127 (49%)	124 (53%)
Unknown	6	0
No. of affected offspring	143	101
Male	73	47
Female	70	54
Mental retardation		
Retarded	103	89
Severe	25 (24%)	73 (82%)
Moderate	30 (29%)	14 (16%)
Slight	16 (16%)	2 (2%)
Severity unknown	32 (31%)	0 (0%)
Not retarded	6	2
Unknown	34	10
Twin pairs (all same sex)	8	2
Discordant	5	1
Concordant	3 (2 unaffected)	1 (1 unaffected)
OFC of parent known (n = 254 & 118)	21	25
OFC of parent ≤3rd %tile	1	4

TABLE III. Geographic Origin and Consanguinity in Ascertained Microcephaly Families

Origin	Total no. of families	Consanguinity			Coeff. of inbreeding
		Yes	No	Unknown[a]	
Vancouver	127	2	88	37	0.0025
American/European	86	1	67	18	0.0029
Asian	11	1	9	1	0.0057
African	2	0	2	2	—
Unknown	28	0	10	18	—
Jerusalem	59	29	30	0	0.0281
Jewish	33	9	24	0	0.0142
Arab	25	20	5	0	0.0475
Other	1	0	1	0	—

[a] Consanguinity status not noted in patient records.

Jerusalem, 33 were Jews, 25 were Arabs, and 1 was from Cyprus.

Consanguinity was observed in both the Vancouver and Jerusalem families; however, as expected, the rates were very different. Among the 90 families for whom consanguinity information was available in the Vancouver sample, only 2 families were inbred. In the Jerusalem sample, 29 of the 59 couples were inbred. When divided by ethnicity, 9 of 33 (27%) Jewish couples and 20 of 25 (80%) Arab couples were consanguineous—a difference that is highly significant ($\chi^2 = 15.8$, 1 df, $P < 10^{-4}$). These data were used to calculate the coefficient of inbreeding in the two samples; the inbreeding coefficients were 0.0025 and 0.0281, respectively—a tenfold difference. As we shall later see, this difference has an impact on the interpretation of the results of our segregation analysis.

Segregation Analyses of the Vancouver Sample

The results of classical segregation analysis are provided in Table IV, and show that although pure dominant and recessive models can be soundly rejected, allowing for the presence of sporadic cases does provide an adequate fit to the family data. Assuming dominant inheritance for microcephaly suggests that 76% of the cases are sporadic ($P = 0.076$), while assuming recessive inheritance implies that 57% of cases are sporadic ($P = 0.220$). Although both models fit the data, the recessive model shows a much better fit to the observations. The general model including sporadics estimated a segregation ratio of 25% with 57% sporadic cases ($P = 0.110$). When a general model is considered, but sporadic cases are assumed absent, the segregation ratio is 11% and provides acceptable fit to the data ($P = 0.119$). If one postulates that all cases of microcephaly are genetic then this gene is highly incompletely penetrant. On the other hand, if non-genetic cases are allowed for, then the best fitting model is one of recessive inheritance with a substantial (57%) fraction of sporadics ($P = 0.110$). Thus, on the basis of fit of estimated parameters to observations, the recessive model with sporadic cases is the most likely inheritance pattern for microcephaly.

When complex segregation analysis was performed on this sample, as shown in Table V, the sporadic and multifactorial models could both be soundly rejected ($\chi^2 = 200.78$, df = 5, $P < 10^{-4}$; $\chi^2 = 20.58$, df = 3, $P = 0.0001$, respectively) when compared to the general mixed model. However, the childhood heritability was estimated as 100%, suggesting strong genetic influences on transmission. The dominant and recessive major gene models fit the data equally well in comparison to the general mixed model ($\chi^2 = 0.43$, df = 3, $P = 0.93$; $\chi^2 = 0.41$, df = 3, $P = 0.94$, respectively), with mutant allele frequencies of 6.0×10^{-5} and 7.5×10^{-3}, respectively. The general mixed model demonstrates recessive inheritance but suggests residual polygenic effects. However, this model is statistically not superior to either major locus model.

Table VI presents the penetrances and proportion of gene carriers for the most parsimonious major gene models identified by complex segregation analysis in the Vancouver sample. Assuming a dominant model, the penetrance is 50%, however, the proportion of gene carriers among affected individuals is estimated to be just 59%, implying that 41% of affected individuals represent sporadic cases of microcephaly. For the recessive model the penetrance was estimated at 86%, while approximately one half of affected individuals would be expected to be sporadic cases of microcephaly. Thus, under either model, 41–51% of cases are sporadic which is slightly lower, but comparable to the 57–76% of sporadic cases estimated from classical segregation analy-

TABLE IV. Classical Segregation Analysis of the Vancouver Sample

Model	P[a]	X	π	χ^2 (df)	P-value
Pure dominant	(0.50)	(0.00)	0.74	55.50 (4)	$<10^{-4}$
Dominant + sporadics	(0.50)	0.76	0.31	6.87 (3)	0.076
Pure recessive	(0.25)	(0.00)	0.52	14.37 (4)	0.006
Recessive + sporadics	(0.25)	0.57	0.29	4.42 (3)	0.220
General	0.11	(0.00)	0.31	5.85 (3)	0.119
General + sporadics	0.25	0.57	0.29	4.39 (2)	0.110

[a] Figures in parentheses indicate parameter value fixed, not iterated.

TABLE V. Complex Segregation Analysis of the Vancouver Sample

Model	d[a]	t	q	h	z	−2 ln L
Sporadic	—	—	—	—	—	−139.87
Multifactorial	—	—	—	1.00[b]	0.30	−320.07
Major gene						
Dominant	(1.00)	3.92	6.0×10^{-5}	—	—	−340.22
Recessive	(0.00)	4.97	0.0075	—	—	−340.24
Mixed	0.00[b]	4.37	0.0240	1.00[b]	1.00	−340.65

[a] Figures in parentheses indicate parameter value fixed, not iterated.
[b] Parameter iterated to a bound.

sis (Table IV). The results of these various analyses thus demonstrate recessive inheritance of microcephaly, although dominant inheritance cannot be completely ruled out.

Segregation Analyses of the Jerusalem Sample

The results of classical segregation analysis are provided in Table VII, and show that dominant inheritance with 29% of the cases being sporadic ($P = 0.634$) fits the family data better than a pure dominant model ($P = 0.068$), although neither could be excluded. The pure recessive model provided a comparable fit to the data ($P = 0.655$). When a recessive model with sporadic cases was fit to the data, the estimate of the proportion of sporadic cases was 0%, thus resembling pure recessive inheritance. The segregation ratios estimated by the general model, with and without the presence of sporadic cases, were 39% and 31%, respectively ($P = 0.712$ and 0.637).

When complex segregation analysis was performed on this sample, as shown in Table VIII, the sporadic and multifactorial models could be soundly rejected ($\chi^2 = 603.15$, df = 5, $P < 10^{-4}$; $\chi^2 = 77.30$, df = 3, $P < 10^{-4}$, respectively) when compared to the general mixed model. The recessive major gene model could also be rejected ($\chi^2 = 8.54$, df = 3, $P = 0.036$), but the dominant model could not ($\chi^2 = 4.34$, df = 3, $P = 0.227$). Thus, the most parsimonious model of inheritance identified by complex segregation analysis for the Jerusalem sample was a dominant major gene model with a mutant allele frequency of 6.2×10^{-5}. However, the mixed model should not necessarily be overlooked for this sample. The mixed model estimated from the data includes a major locus with a rare, additive mutant allele in combination with polygenes. Such a model could provide evidence for multiple inherited factors conferring susceptibility to microcephaly in the Jerusalem sample.

Table IX presents the penetrances and proportion of gene carriers for the major gene models identified by complex segregation analysis in the Jerusalem data. Assuming a dominant model, the penetrance is 66%, with an estimated 82% of gene carriers among affected individuals, corresponding to just 18% of affected individuals having sporadic disease. For the recessive model the penetrance was estimated at 100%, while 27% of affected individuals would be expected to be sporadic cases. Thus, under the dominant model, 18% of cases are sporadic which is lower than, but comparable to, the 29% of sporadic cases estimated from classical segregation analysis (Table VII). The estimate of proportion of gene carriers for the recessive model is quite different from the two analyses: classical segregation analysis predicted no sporadic cases, while complex segregation analysis predicted 27% of the cases as sporadic.

The simple and complex segregation analyses gave similar and consistent results for both samples. The Vancouver data fit a recessive mode of inheritance the best, but a dominant model could not be rejected. Furthermore, both models predicted the existence of sporadic cases within the Vancouver sample. The Jerusalem data demonstrated dominant inheritance with additional sporadic cases.

DISCUSSION

Microcephaly is a heterogeneous genetic disorder since, based on inheritance patterns in families, and now the results of segregation analyses, different genes, both recessive and dominant, are susceptibility factors. In addition, environmental factors are an important contributor as well, since several known teratogens have been identified.

The results of our segregation analyses on the Vancouver sample indicated that an estimated 43–49% of affected individuals have microcephaly due to homozygosity for recessive mutant alleles, with the remainder being sporadic cases. The penetrance of the recessive homozygote is very high (86%). This suggests a simple model of inheritance for microcephaly, i.e., a mixture of mendelian recessives with non-genetic cases. Segregation analysis cannot determine whether the recessive alleles are all at a single locus or not; this must await further genetic mapping. Nevertheless, a dominant model of inheritance, also with sporadic cases, could not be rejected; such a postulated dominant mutation(s) would have low (50%) penetrance. The analysis of the Jerusalem sample is instructive in choosing between these two models of inheritance.

TABLE VI. Penetrance and Proportion of Gene Carriers in the Vancouver Sample

	Penetrance of genotype			Genotype proportions among affected		
Model	P(aff|AA)	P(aff|Aa)	P(aff|aa)	P(AA|aff)	P(Aa|aff)	P(aa|aff)
Dominant	4×10^{-5}	0.50	0.50	0.41	0.59	2×10^{-5}
Recessive	5×10^{-5}	5×10^{-5}	0.86	0.51	<0.01	0.49

TABLE VII. Classical Segregation Analysis of the Jerusalem Sample

Model	P[a]	X	π	χ^2 (df)	*P*-value
Pure dominant	(0.50)	(0.00)	0.58	10.28 (5)	0.068
Dominant + sporadics	(0.50)	0.29	0.50	2.56 (4)	0.634
Pure recessive	(0.25)	(0.00)	0.46	3.29 (5)	0.655
Recessive + sporadics	(0.25)	0.00[b]	0.46	3.29 (4)	0.511
General	0.31	(0.00)	0.49	2.13 (4)	0.712
General + sporadics	0.39	0.18	0.49	1.70 (3)	0.637

[a] Figures in parentheses indicate parameter value fixed, not iterated.
[b] Parameter iterated to a bound.

In the Jerusalem sample, both segregation analyses showed that a dominant model provided the best fit to the data, with 18–29% of the cases being sporadic, although classical segregation analysis could not rule out a recessive mode of inheritance. Further, the recessive model was only marginally rejected ($P = 0.036$) by complex segregation analysis; this result should be evaluated in the light of the multiple comparisons made. The finding of dominant inheritance as a parsimonious explanation of the Jerusalem segregation data can be interpreted in two ways: a) in some families, microcephaly is inherited as a dominant trait, and b) the sample, ascertained from a genetic counseling service, was overrepresented by multi-case families. The first interpretation is possible since one family in the Jerusalem sample showed probable dominant inheritance. If dominant inheritance is responsible in a substantial number of families then we need to seek explanations as to why the gene is so non-penetrant in the parents yet highly penetrant in the offspring. This characteristic is clearly indicated by the complex segregation analysis in both samples, where the offspring and parental heritability is 100% and 30%, respectively (Tables V and VIII). This is more reminiscent of recessive inheritance with a small contribution from dominant forms. The second interpretation is also plausible since there is a difference between the samples in the frequency of affected offspring beyond the first. In the Vancouver and Jerusalem samples there were 12 and 23 families with more than one affected child; there were 28 and 65 affected children in these families, respectively. Additionally, the first affected child was a proband in $^{8}/_{12}$ and $^{7}/_{23}$ families in the Vancouver and Jerusalem samples, respectively. Conversely, in $^{4}/_{12}$ (33%) of the families in the Vancouver sample and in $^{16}/_{23}$ (70%) of the families in the Jerusalem sample either the proband was not the first affected child or all affected children were probands. These results indicate that, in Jerusalem, a greater proportion of families were referred to the genetic counseling service only after they had more than one affected child. This higher proportion of families with multiple affected children would falsely inflate the segregation ratio, implying a genetic model which appears to be dominant with reduced penetrance in the parental generation. We consider this to be the more likely explanation.

A segregation analysis of 118 microcephalic patients and their families, ascertained in India, was performed by Sujatha et al. [1989]; 61 (52%) of these nuclear families were consanguineous—this rate is similar to the 49% consanguinity rate observed in the Jerusalem sample. These authors performed segregation analysis after dividing the sample into primary (n = 61) and secondary (n = 57) microcephaly. In the group with primary microcephaly, the segregation ratio was estimated to be 25% in the consanguineous families and 13% in the nonconsanguineous families. A simple interpretation of these results would be that microcephaly is inherited in a recessive fashion, and that a substantial number of sporadic cases (~50%) account for the lower segregation ratio among the nonconsanguineous families. These results are fully compatible with our observations on the Vancouver sample.

Classical segregation analysis estimated the segregation ratio as 11% and 31% in the Vancouver and Jerusalem samples, respectively. In the Vancouver sample, the 11% value is compatible with recessive inheritance in 43% of families, the remaining 57% of cases being sporadic (.25 × .43 = .11). The higher segregation ratio in the Jerusalem sample may be a reflection of the high consanguinity rate observed in this sample. The coefficient of inbreeding for the Israeli sample (0.0281) was higher than that in the respective Jewish and Arab-Israeli populations. Among Eastern and Sephardi Jews, first cousin matings occur with a frequency of 3–18%, while among Ashkenazi Jews the frequency of first cousin matings is 0.3–3%. Among Israeli-Arabs, 29–40% of matings are consanguineous [Cohen, 1992]. In the Jerusalem sample, 8 of 25 East-

TABLE VIII. Complex Segregation Analysis of the Jerusalem Sample

Model	d[a]	t	q	h	z	−2 ln L
Sporadic	—	—	—	—	—	632.33
Multifactorial	—	—	—	1.00[b]	0.30	106.48
Major gene						
Dominant	(1.00)	4.53	6.2×10^{-5}	—	—	33.52
Recessive	(0.00)	8.27	0.0085	—	—	37.72
Mixed	0.52	8.11	8.0×10^{-5}	1.00[b]	0.29	29.18

[a] Figures in parentheses indicate parameter value fixed, not iterated.
[b] Parameter iterated to a bound.

TABLE IX. Penetrance and Proportion of Gene Carriers in the Jerusalem Sample

	Penetrance of genotype			Genotype proportions among affected		
Model	P(aff|AA)	P(aff|Aa)	P(aff|aa)	P(AA|aff)	P(Aa|aff)	P(aa|aff)
Dominant	2×10^{-5}	0.66	0.66	0.18	0.82	3×10^{-5}
Recessive	3×10^{-5}	3×10^{-5}	1.00	0.27	<0.01	0.73

ern and Sephardic Jewish families (32%), 1 of 8 Ashkenazi families (13%) and 20 of 25 Arab families (80%) were inbred. These results indicate a greater rate of consanguinity in the families of microcephalic patients than in controls; this would support the hypothesis that recessive alleles are important in the causation of microcephaly in the Jerusalem sample. However, the segregation ratio for a recessive disorder cannot exceed 25%. The finding of a 31% segregation ratio implies the existence of dominant forms or persistent over-sampling of multiplex families, or both. Pseudodominance due to the mating of a heterozygote with a nonpenetrant mutant homozygote should also be considered for this population. The relatively high background level of inbreeding observed in Israel, in combination with higher-than-expected rates of consanguinity observed in our sample population, could provide a setting where this type of mating is not only possible, but probable. Such a phenomenon could contribute another explanation for why the segregation ratio would be inflated above the expected rate of 25% for a recessive disorder.

The apparently different modes of inheritance for microcephaly, recessive with sporadics in the outbred Vancouver sample and dominant with sporadics in the Jerusalem sample, can be reconciled in a different way. Although much of the current thinking in human genetics is in the classification of phenotypes as either dominant or recessive, most phenotypes have a complex mode of inheritance [Chakravarti and Lander, 1990]. This complexity often arises from the multigenic inheritance pattern of diseases, where a major susceptibility locus is modified by one or more genes [Haldane 1941], as we have recently demonstrated for the inheritance of Hirschsprung disease [Badner et al., 1990; Puffenberger et al., 1994a]. Alternatively, the effect of a mutation may be such that homozygotes have much greater risk than heterozygotes, as we have recently shown for a endothelin-B receptor mutation in Hirschsprung disease [Puffenberger et al., 1994b]. Thus, the frequency of mutant gene(s) and the degree of inbreeding within a population may determine the apparent mode of inheritance for a phenotype, which may vary between populations. The results of our segregation analysis clearly suggests the action of both recessive alleles and the occurrence of environmental sporadic cases in microcephaly. It is now imperative that this hypothesis be tested directly by linkage studies in multiplex sibships. Molecular genetic analysis will allow us to determine the precise nature and number of the loci involved in the etiology of microcephaly. The purposeful sampling of multiply affected sibships not only provides linkage evidence, but also has the advantage of sampling genetic cases.

Irrespective of the actual mode of inheritance of microcephaly, our family data are useful for risk prediction in proband families based on genetic models. The recurrence risk for microcephaly, in various sibship sizes and for varying degrees of familiality, are presented in Table X. The results are classified by mode of inheritance and for each of the Vancouver and Jerusalem samples. It is apparent that the recurrence risks in each case are greater in the Jerusalem, than in the Vancouver, sample. However, these risks are almost equal for either genetic model assumed. As the family data do not allow us to unequivocally distinguish modes of inheritance, they do not affect the recurrence risk either. The genetic risks are all between 5% and 25%, depending on family history, and are all significantly greater than the population risk of 0.01%. Thus, the relative risks for family history are between 500 and 2,500, indicating a strong genetic effect.

ACKNOWLEDGMENTS

The authors would like to thank Jillian Blaschak for technical assistance. This work was supported in part by the British Columbia Children's Hospital Foundation (J.G.H.) and by NIH grants HD28088 (A.C.) and HG00344 (A.C.).

TABLE X. Genetic Risk of Affection in Microcephaly

Family type		Study sample			
		Vancouver		Jerusalem	
Sibship size	No. affected	Recessive	Dominant	Recessive	Dominant
1	1	0.05	0.05	0.09	0.10
2	1	0.06	0.06	0.11	0.11
	2	0.15	0.17	0.17	0.22
3	1	0.06	0.06	0.11	0.10
	2	0.16	0.19	0.19	0.25
	3	0.16	0.19	0.19	0.25

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